PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference JP802710-142	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. **PCT/NZ2004/000247**	International filing date *(day/month/year)* 11 October 2004	Priority date *(day/month/year)* 9 October 2003

International Patent Classification (IPC) or national classification and IPC

Int. Cl.[7] C07K 14/415

Applicant

NEW ZEALAND INSTITUTE FOR CROP & FOOD RESEARCH LIMITED et al

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. [X] *(sent to the applicant and to the International Bureau)* a total of **2** sheets, as follows:
      - [X] sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      - [ ] sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.

   b. [ ] *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or table related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

[X]	Box No. I	Basis of the report
[ ]	Box No. II	Priority
[ ]	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
[ ]	Box No. IV	Lack of unity of invention
[X]	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
[ ]	Box No. VI	Certain documents cited
[X]	Box No. VII	Certain defects in the international application
[ ]	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
3 May 2005	15 September 2005
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **R.L. POOLEY** Telephone No. (02) 6283 2242

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/NZ2004/000247

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:

   ☐ international search (under Rules 12.3 and 23.1 (b))

   ☐ publication of the international application (under Rule 12.4)

   ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements** of the international application, this report is based on (*replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report*):

   ☐ the international application as originally filed/furnished

   ☒ the description:

   pages **1-19** as originally filed/furnished
   pages* received by this Authority on with the letter of
   pages* received by this Authority on with the letter of

   ☒ the claims:

   pages as originally filed/furnished
   pages* as amended (together with any statement) under Article 19
   pages* **20-21** received by this Authority on **28 July 2005** with the letter of **28 July 2005**
   pages* received by this Authority on with the letter of

   ☒ the drawings:

   pages **1/4-4/4** as originally filed/furnished
   pages* received by this Authority on with the letter of
   pages* received by this Authority on with the letter of

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing (*specify*):
   ☐ any table(s) related to the sequence listing (*specify*):

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

   ☐ the description, pages
   ☐ the claims, Nos.
   ☐ the drawings, sheets/figs
   ☐ the sequence listing (*specify*):
   ☐ any table(s) related to the sequence listing (*specify*):

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-19	YES
	Claims		NO
Inventive step (IS)	Claims	1-19	YES
	Claims		NO
Industrial applicability (IA)	Claims	1-19	YES
	Claims		NO

2. Citations and explanations (Rule 70.7)

The following documents were cited in the International Search Report:

D1 - WO 2000/017328 A2

D2 - WO 2002/042321 A2

D3 – WO 2001/ 027152 A1

D4 – Chen et al, Biochemistry, Vol. 41, 2002, p 7391-7399

D5 – Takahashi et al, Structure with Folding & Design, Vol. 8 (9), 15 September 2000, p 915-925

D6 – Takahashi et al, Chembiochem: A European Journal of Chemical Biology, Vol. 3(7), 2 July 2002, p 637-642

NOVELTY (N) and INVENTIVE STEP (IS)

The above documents all disclose the in vitro production of amyloid fibrils from sources of protein that could be considered "heterogeneous". However they do not disclose or suggest the formation of amyloid fibrils from a wheat protein source as now defined. Accordingly claims 1-19 are novel and inventive over the disclosures of documents D1-D6.

Document D1 discloses non-naturally occurring amyloid fibrils derived from mixtures of protein from different sources (see page 3) including high molecular weight sources such as bovine insulin. It also discloses the formation of the fibrils via the process steps of claims 10-16 (eg using urea as a denaturing compound) and using a protein seed (see page 5). However, it does not disclose or suggest the formation of amyloid fibrils from a wheat source as now defined. Accordingly claims 1-19 are novel and inventive over document D1.

Document D2 also discloses amyloid fibrils formed from different peptides and thus uses a "heterogeneous" source of protein as a starting material. It also discloses the use of insulin as one of the proteins forming the fibril (see page 4), the use of the fibril as a biomaterial (see page 3) and the use of a protein seed in formation of the fibril. However, it does not disclose or suggest the formation of amyloid fibrils from a wheat source as now defined. Accordingly claims 1-19 are novel and inventive over document D2.

Document D3 discloses the production of amyloid fibrils from a variety of polypeptide sources including synthetic and non-natural sources, and the use of these fibrils to release other polypeptide molecules (see page 26). These sources of proteins would seem to be "heterogeneous". Document D3 also discloses the use of urea in a nucleation process to produce the fibrils. However, it does not disclose or suggest the formation of amyloid fibrils from a wheat source as now defined. Accordingly claims 1-19 are novel and inventive over document D3.

Document D4 discloses a variety of synthetic polyglutamine peptides that appear to form amyloid fibrils. As the

Continued in Supplemental Box

Box No. VII Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

The description does not comply with Rule 11 due to the inclusion of drawings at page 8.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.

Continuation of: Box V

peptides are synthetic, they would seem to derive from a "heterogeneous" source. Document D4 also discloses the formation of amyloid fibrils using the steps of claims 11-16 (see page 7396) and the use of aggregate seeds (see page 7394). However, it does not disclose or suggest the formation of amyloid fibrils from a wheat source as now defined. Accordingly claims 1-19 are novel and inventive over document D4.

Documents D5 and D6 disclose the formation of amyloid fibrils using synthetic peptides that seem to fall within the scope of a "heterogeneous" source of protein. However, they do not disclose or suggest the formation of amyloid fibrils from a wheat source as now defined. Accordingly claims 1-19 are novel and inventive over documents D5 and D6.

NDUSTRIAL APPLICABILITY (IA)

Claims 1-19 are considered to possess industrial applicability.

Claims

1. A method of amyloid fibril formation using a heterogeneous source of protein as a starting material, and wherein the protein source is from a wheat source or a wheat protein sequence from a microbiological expression system.

2. A biomaterial containing amyloid fibrils made according to claim 1.

3. A method of producing amyloid fibrils from a high molecular weight, heterogeneous source of wheat protein.

4. A method according to claim 3 in which the wheat protein is from a microbiological expression system.

5. A method according to claim 3 or claim 4 wherein the starting material is an SDS-soluble wheat protein fraction.

6. A method according to claim 3 or claim 4 wherein the starting material is an SDS-insoluble wheat protein fraction.

7. Amyloid fibrils produced by the method of any one of claims 1 or 3-6.

8. A method of producing amyloid fibrils derived from wheat, comprising:
   a) Producing wheat protein, crudely fractionated from a milled flour;
   b) Separating a heterogeneous protein mixture on the basis of solubility;
   c) Obtaining protein solutions containing a broad range of proteins of varying molecular weights and compositions;
   d) Incubating these fractions at moderate temperatures, typically in the presence of specific compounds known to destabilise a protein's structure, to induce the formation of amyloid.

9. Amyloid fibrils produced by the method of claim 8.

10. A method according to any one of claims 1, 3-6, or 8 wherein the method is performed *in vitro*.

PCT/NZ2004/000247
Received 28 July 2005

11. A method according to any one of claims 1, 3-6, 8 or 10 wherein a denaturing compound is added to induce the formation of amyloid-like structures.

12. A method according to claim 11 wherein the denaturing compound is one or more of urea, a thiol containing reductant (e.g. dithiothreitol (DTT)), or an acid (e.g. H_2SO_4, HCl).

13. A method according to claim 12 wherein the pH range is 2-7.5, preferably 5-7.5.

14. A method according to claim 12 or 13 wherein the temperature range is 20-70°C, preferably about 50°C.

15. A method according to any one of claims 11-14 wherein the denaturing compound is incubated with the protein source at 25°C for up to 105 days.

16. A method according to any one of claims 11-14 wherein the denaturing compound is incubated with the protein source at 37°C for up to 105 days.

17. A method according to any one of claims 8 or 10-16 wherein the incubated protein is substantially a wheat protein sequence from a microbiological expression system.

18. A method according to any one of claims 1, 3-6, 8 or 10-17 wherein an extraneous amyloid fibril (e.g. insulin fibril) is added as a "seed" to include amyloid fibril formation in the wheat protein treatments.

19. Amyloid fibrils produced by the methods of any one of claims 10-18.